A CULTURE CHAMBER FOR THE STUDY OF MYCORRHIZAE

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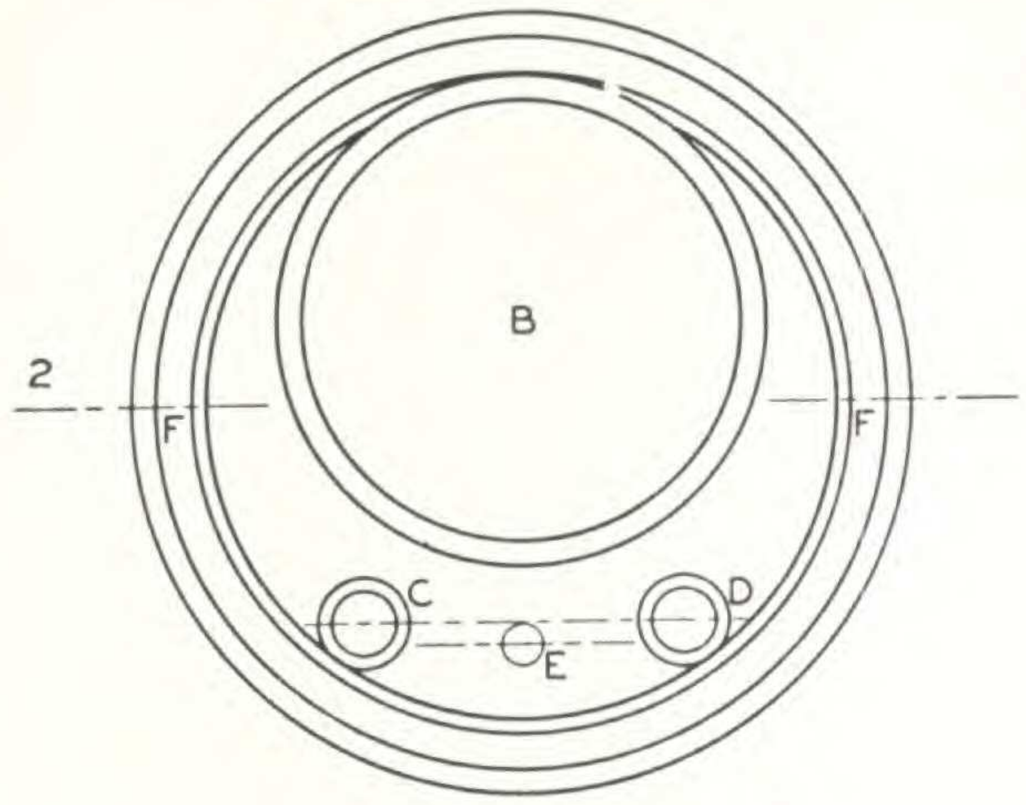
With two text figures and plate 118

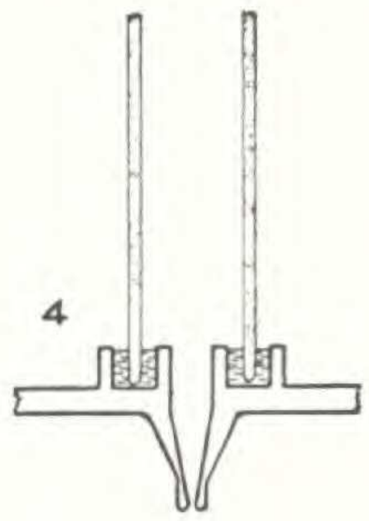
THE EXACT NATURE of mycotrophic relationships still remains unknown. A first essential of investigation in this field is the development of suitable experimental methods. Among these an outstanding requirement is that of establishing and maintaining pure cultures. The usual apparatus for this purpose is cotton stoppered flasks but the physical environments in these are optimal for neither host nor fungus and extraneous factors that affect the accuracy of results are not eliminated. A culture chamber that meets these requirements has been developed by the writer and is described below.

Factors which affect unfavorably the accuracy of experiments in the apparatus commonly employed include: (1) excessive humidities which promote aërial growth of fungi which in nature are confined to roots only (Melin, 1925; Masui, 1927; Rayner, 1925, 1930; McArdle, 1932; Hatch and Hatch, 1933), (2) increased partial pressures of carbon dioxide in inoculated cultures produce corresponding increases in plant growth but in a manner wholly unrelated to mycotrophy, (3) accumulation of products (in some cases toxic) of fungal metabolism and of unabsorbed ions in substrates that cannot be changed (Melin, 1925), (4) saturated substrates—when fine quartz sand is employed (Rayner, 1930; McArdle, 1932), (5) low radiation intensities resulting in low rates of carbohydrate synthesis.

It is generally believed that if mycorrhizae are beneficial, they must be *accessory* mechanisms only, and by most investigators on this subject they are thought to be concerned with acquisition of nitrogen from infertile substrates. In the closely allied field of nitrogen-fixing, bacterial root-nodules it is known that these accessory mechanisms are called forth under internal conditions of wide carbohydrate/nitrogen ratio only (Fred and Wilson, 1934). The necessity of maintaining rapid rates of carbohydrate synthesis by increasing partial pressures of CO_2, by high radiation intensities, or by both, is evident.

Finally, to be conclusive the data should be quantitative and this involves the growing of large numbers of plants—a task of large proportions in ordinary chambers (McArdle, 1932).





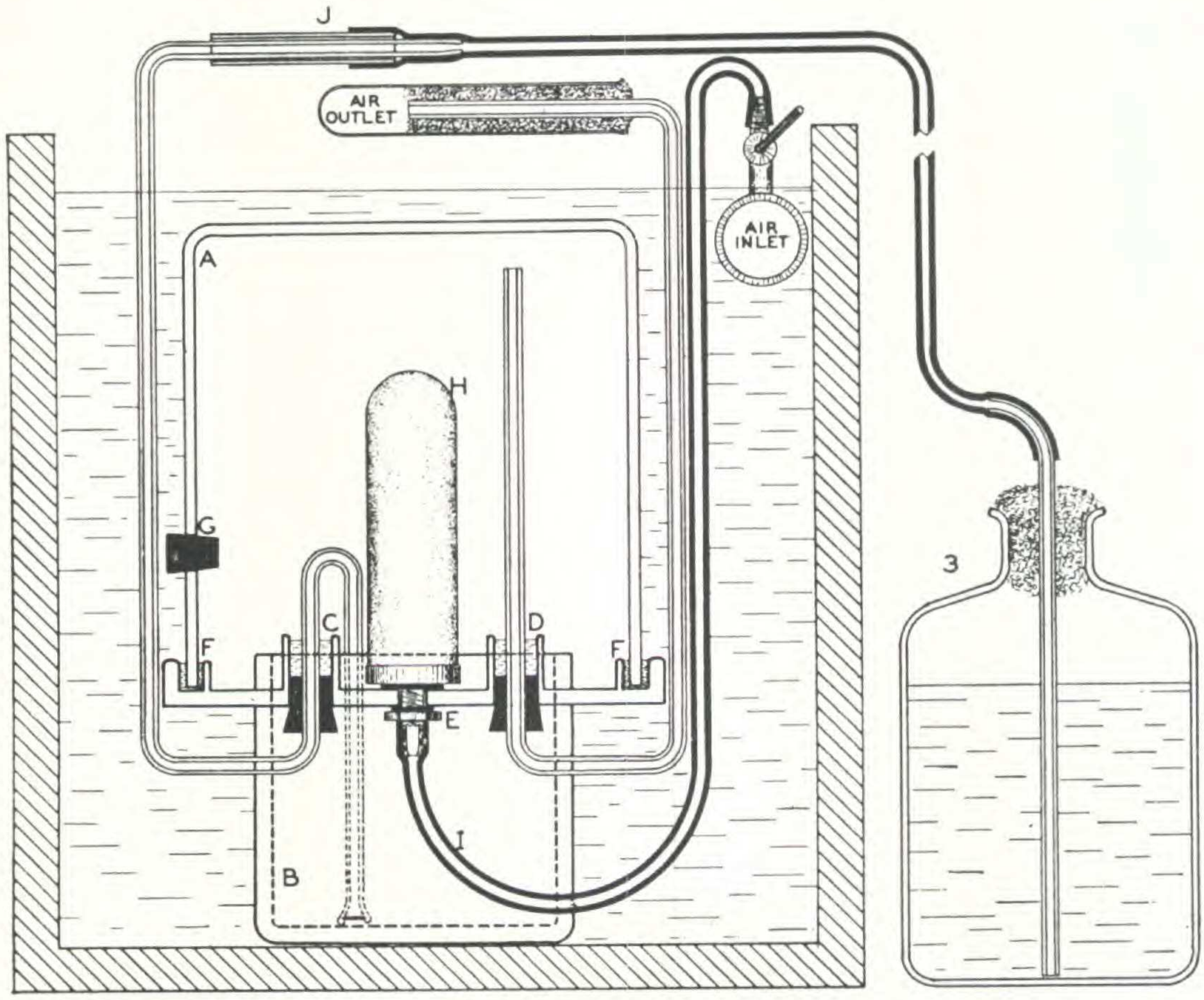


FIGURE 1. Pure culture chamber with 10 × 10⅛ inch bell jar. 1. Schematic drawing of intact chamber in water bath. 2. Plan of porcelain base. 3. Nutrient flask. 4. Structure of alternative base for open-end filter. (Drawings by Robert Ward and the writer.)

To avoid these inaccuracies an apparatus has been developed which facilitates the following: (1) continuous aëration (control of gas composition and moisture content), (2) periodic flooding and aërating of substrate, (3) frequent changes of nutrient or water, (4) exposure to direct solar or artificial radiations of the highest intensities, (5) temperature control, by means of a constant temperature water bath, and (6) indefinite freedom from contamination.

The plant chamber of this apparatus consists of a glazed clay base (my early models had copper bases—see plate 119) through which are inserted all connections and over which is cemented a Pyrex cylinder jar (Fig. 1,[1.A]). The base possesses a substrate well B and a circular platform through which are three openings C, D, and E. Openings C and D are identical, having inside diameters of approximately 2 cm. and heights of 4 to 5 cm. Through these openings heavy-walled pyrex tubes are inserted; one (through C), for changing nutrients, extends to the bottom of the substrate well; the other (through D), for air escape, extends within the chamber to a position near the top of the bell jar opposite the air inlet filter H (in the figure shown in a vertical position). Opening E is for inserting the air filter H, and may be of two types: a plain hole through which a Berkefeld, grade V, water filter is sealed with rubber washers, as shown in the main drawing; or a trough is built around the filter opening and an open base filter is cemented into it during sterilization (Fig. 1,[4]). (The open base filter and suitable chamber base are much less expensive.)

The circular "platform" possesses a trough F around its margin, into which the cylinder jar is cemented. As indicated in Fig. 1, [2.B], the substrate-well is off-center to give space for the three openings C, D, and E. In existing samples of the clay base chamber the glaze has not been wholly satisfactory; accordingly, a pyrex cylinder jar of small size (6 × 6 inches) has been wedged inside of the well to serve as the actual substrate container (see plate 118, which shows this jar in the metal base chamber).

The upper half of the chamber, as indicated, is an inverted pyrex cylinder jar with a 17 mm. opening G through its wall for inoculating and for introducing germinating seeds (approximately 75 mm. above the bottom rim).

The position of the nutrient changing, and air outlet tubes (extending through C and D) on the outside of the chamber is determined by convenience and the method used in maintaining the chamber at a constant temperature. Their positions in Fig. 1,[1] are adapted for complete immersion of the chamber in water.

Sterilization of the chambers may be by steam or by hot air, each of which involves separate consideration.

With steam sterilization, either a Berkefeld filter with rubber seals or the open ended type may be employed, and also rubber stoppers for holding tubes C and D in place as shown in Fig. 1,[1]. Cotton is placed in the inoculation opening of the glass jar and over the outside ends of the tubes through C and D. The base, with sand substrate, and the cylinder jar are then sterilized separately for three hours at one atmosphere; the pressure is allowed to escape quickly; DeKotinsky cement, which has been previously stretched to a suitable thinness, is placed in the spaces above the rubber stoppers in C and D and completely around the trough E, into which the glass jar fits, and also in the filter trough (Fig. 1,[4]) when the open-base filter is used. The intact chamber is then ready for immediate resterilization for 25-45 minutes. Such heating periods are sufficient for killing any organisms which enter during these manipulations and yet are not so long that the strength of the cement is destroyed. A perfect seal is obtained during this final sterilization and by ordinary careful handling the chamber may be kept for months or perhaps years without danger of contamination. Since DeKotinsky cement is slightly soluble in water, all surfaces that come in contact with water in the constant temperature bath should be protected, preferably with picein.

With hot air sterilization, rubber connections must be replaced by cork or other material. The Berkefeld filter is also awkward in this method and the open-base filter (Fig. 1,[4]) must replace it. An advantage of hot air sterilization is that picein cement, which is less expensive than DeKotinsky and also insoluble in water, may be employed. In steam sterilization picein is not suitable, since its specific gravity is less than water and condensing steam prevents the sealing of joints. If chambers of the larger sizes, for agricultural plants, are used (30 $\times$ 14 inches) hot air is most suitable, unless large soil sterilizers are available.

Surface sterilized seed and fungal cultures are inserted through the inoculation opening G while the chamber is in a transfer room. This opening is then sealed with a rubber stopper and picein cement or with the latter alone.

Aëration of the chamber is accomplished preferably by air pressure, but precautions should be taken that such air is free of oil and other impurities and that a proper humidity is maintained (see Shelford, 1925, for suitable equipment). Rates of air passage up to several liters per minute are obtained by Berkefeld 5 $\times$ 15 cm. V filters with pressures of 3-5 cm. of mercury. The open-base filters are less porous and

greater pressures are required for comparable air passage. A rubber tube I should be cemented to the base of the filter-opening before immersion.

The nutrient reservoir consists of a 4-liter, or larger, pyrex or Jena glass bottle with a cotton stopper through which is inserted a glass tube (Fig. 1,[3]). Heavy walled rubber tubing connected to this glass tube extends over a 12 mm. tube of 10 cm. length (Fig. 1,[1,J]). After the culture flask and contained nutrient or water are sterilized, this nutrient changing tube and the tube passing through C are thoroughly flamed and brought together so that the smaller tube extends a few centimeters into the larger one of the nutrient flask (J). After cooling to a point where the rubber will not be injured, they are firmly connected. Except for the latter mode of connecting the nutrient supply to the culture chamber, this siphon tube method of changing nutrients in pure culture was developed by Dr. K. D. Doak at the University of Pennsylvania, only an abstract of whose work is now available (Doak, 1934). In its present form it has been regularly employed by Dr. Doak as well as by the writer for several years without a single contamination being traced to its use.

To flood the substrate the siphon is started by cupping one's hand over the cotton stopper of the nutrient flask and blowing. Subsequent raising and lowering of the nutrient flask floods and drains the substrate. The nutrient supply flask may be disconnected (at J) with entire safety and replaced by a fresh nutrient or water supply at any convenient intervals. With exposure to direct solar radiation the rubber tubing should be replaced every few months.

In early experiments air cooling of the chambers during exposure to direct solar radiation proved to be inadequate. The subsequent combination of air cooling and water cooling (of the substrate well only), although fairly effective, was difficult to handle and air temperatures varied. Finally, complete or partial immersions of the chambers in an approximately constant temperature water tank proved to be entirely satisfactory (Fig. 1,[1]). By this method high radiation intensities, both artificial and solar, are usable. The most exacting requirements of temperature control are also possible.

Standard, heavy walled, pyrex cylinder-jars are available in sizes ranging from 6 × 6 in. up to 12 × 24 in., with many intergradations. Agricultural plants of comparatively large statures (potatoes, tomatoes, grains, etc.) may therefore be grown to maturity in chambers of this design under pure culture conditions and in optimum environments.

The chamber has the disadvantage that roots may not be observed during the course of the experiment. An all glass chamber of the type shown in Fig. 2,D, possessing a flattened substrate tube, has been con-

structed to supplement the lack of this feature in the larger chamber. These chambers are made of Florence flasks of any desirable size. By starting plants in them simultaneously with those in the large chamber, and under the same nutrient conditions, the probable course of development of mycorrhizae in the larger chambers may be predicted. This chamber is also immersed in the water bath. Although air change is not

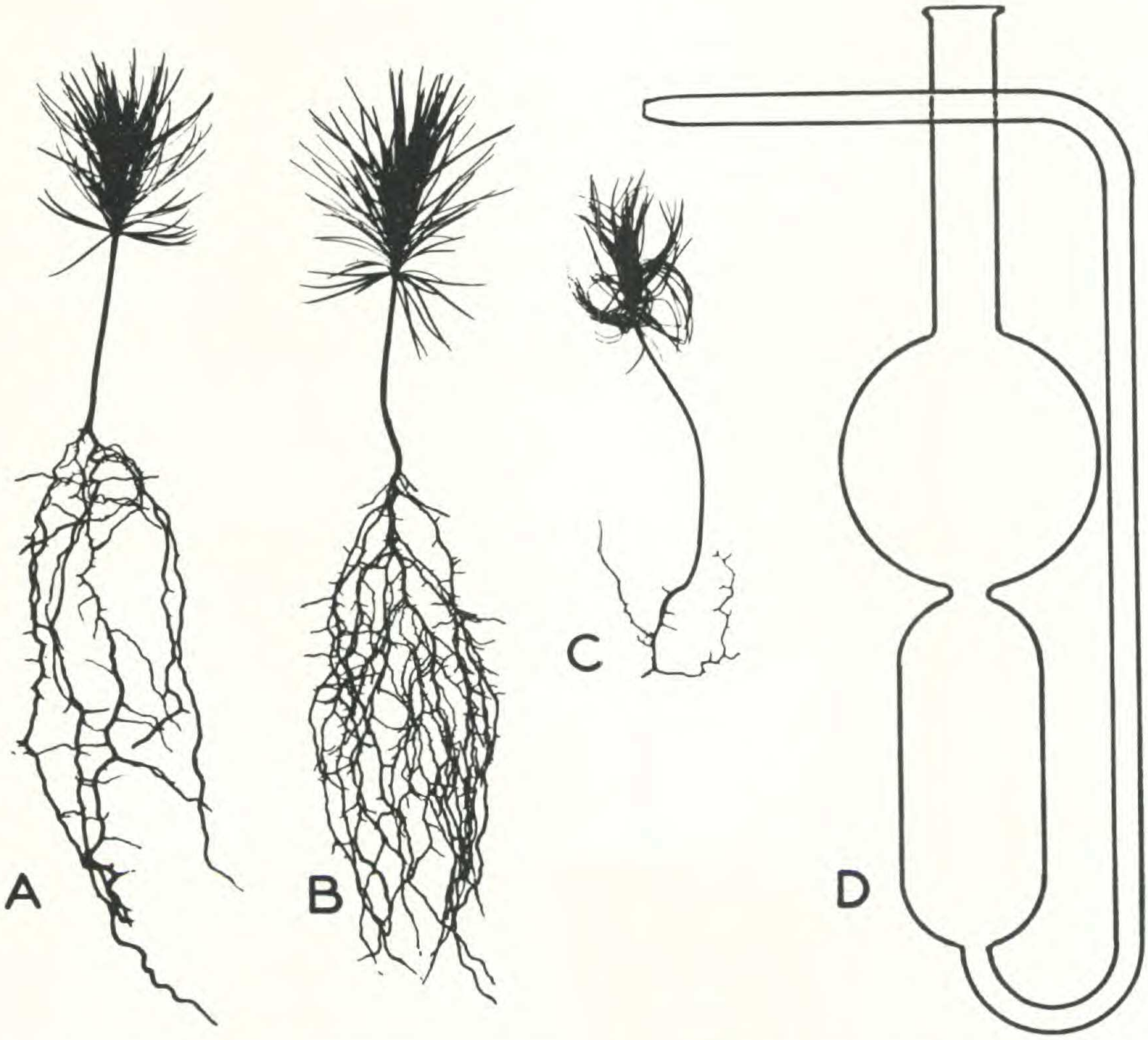


FIGURE 2. Appearance of average seedlings of *Pinus Strobus* grown in sand culture: (A) out of doors (age 3 months), (B) in aërated, nutrient changed pure culture chambers with direct solar radiation and air cooling (age 3 months, February to May 1933) and (C) in Erlenmeyer flasks for 6 months with indirect radiation (Hatch and Hatch 1933). (D) Outline of supplementary culture chamber for observation of roots during course of experiment. Sand substrate fills the flattened tube below the constriction. Glass wool placed in the bottom prevents substrate from moving into nutrient changing tube.

continuous the siphoning of nutrients in and out facilitates a material improvement in the aërial environment over ordinary enclosed flasks.[1]

The types of tree seedlings (*Pinus Strobus* L., 3½ months old) that may be grown in the large chambers are shown in Fig. 2,B. They are quite comparable in size, root shoot ratio, root development, and succulence to those grown in open cultures under direct solar radiation out of doors (Fig. 2,A). An average seedling 6 months old grown in Erlenmeyer flasks is shown in Fig. 2,C.

Development of this apparatus was commenced during the writer's period of study with Professor E. Melin in the mycological laboratories of the Royal Forest Academy, Stockholm, Sweden, in 1930 as Fellow of the American Scandinavian Foundation. The work was continued with the United States Forest Service at the Boyce-Thompson Institute for Plant Research and was finally completed at Harvard University, where the greater part of the work was done. The writer is grateful for the facilities and support of these institutions and to their staffs for advice.

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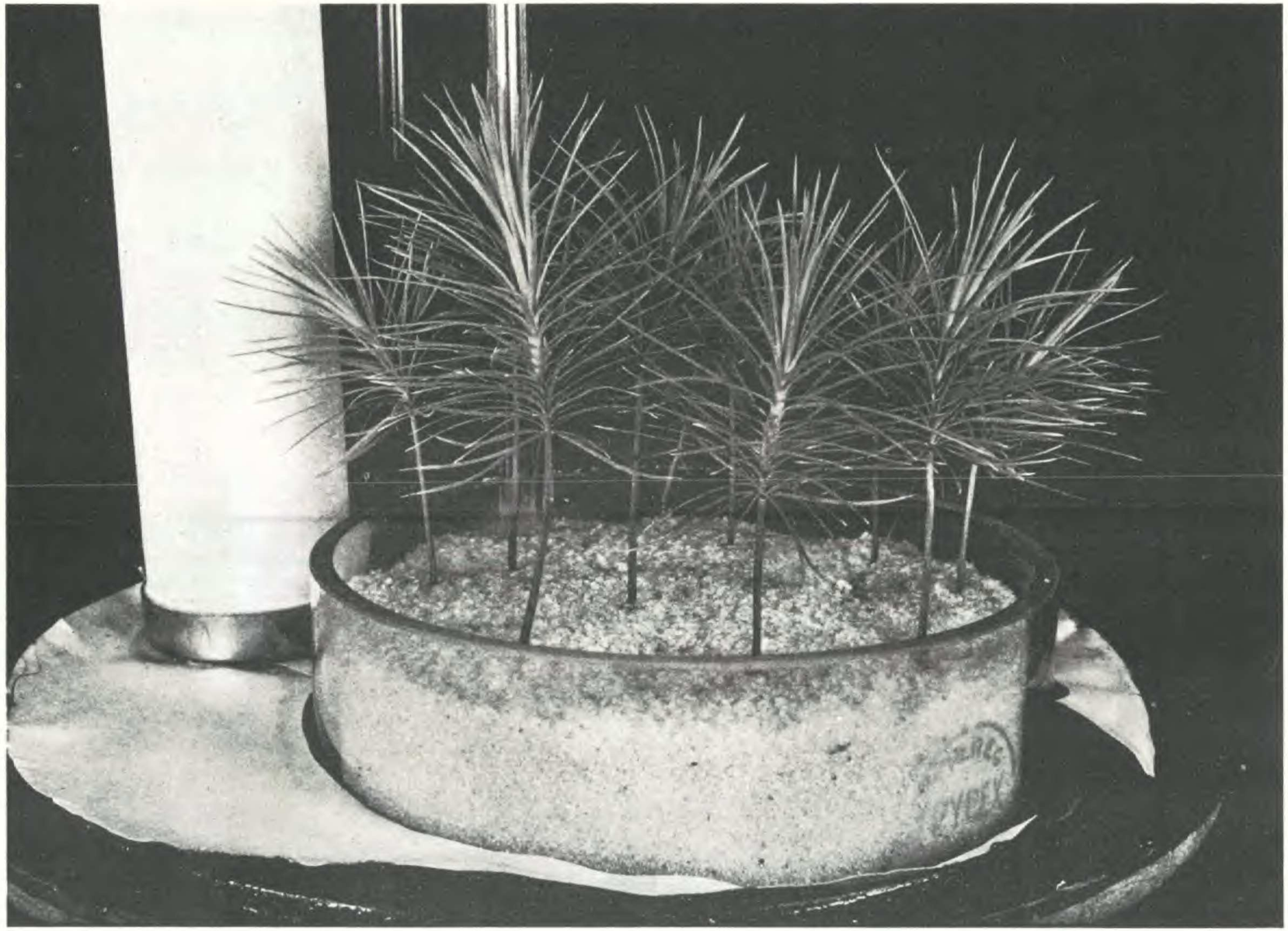
[1]Both chambers complete with all accessories (cement, tubes, stoppers, filters, etc.) are supplied by MacAlaster-Bicknell Co., Cambridge, Mass. Bases for the 10-inch-bell jars are in stock; all other sizes are made to order.



SEEDLINGS OF PINUS STROBUS GROWN IN THE CULTURE CHAMBER